EPITOPE-SPECIFIC REGULATION OF MEMORY B-CELL EXPRESSION*

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SUMMARY

IgG antibody responses to individual epitopes on complex antigens can fail despite the presence of fully competent populations of memory B-cells, ample carrier-specific help and the normal production of IgG antibody responses to other epitopes on the same antigen. These response failures reveal the existence of an "epitope-specific" regulatory system that selectively controls the expression of memory B-cells in antibody responses to hapten-carrier conjugates and other complex antigens.

Our earlier B-cell studies describe successive stages in memory B-cell development and consider the potential role(s) that IgD receptors on "early" memory B-cells play in determining *in situ* primary and anamnestic response characteristics.[1-5] Our more recent work, however, shows that an Igh-restricted regulatory system also plays a major role in defining such response characteristics.[6-12] This previously unrecognized system, which controls memory B-cell expression (rather than development), selectively regulates IgG antibody production to each of the individual epitopes on T-dependent antigens such as DNP-KLH and DNP-CGG (the DNP hapten on keyhole limpet hemocyanin and chicken gamma globulin, respectively)

We have shown that this system can be induced to specifically suppress IgG2a anti-DNP responses to DNP-KLH without interfering with primary or secondary antibody responses to the KLH epitopes on the same molecule. Furthermore, it can be induced to specifically suppress IgH-1b allotype responses to all DNP-KLH epitopes without interfering either with other allotype and isotype responses to DNP-KLH or with Igh-1b responses to other antigens in the same animal. Thus, under conditions where memory development is optimal, this highly versatile regulatory mechanism is key to determining the amount, specificity, affinity and Igh isotype/allotype representation of IgG antibody responses.

Since we have recently published a full description of this Igh-restricted "epitope-specific" system, we have chosen to briefly outline its properties here using a somewhat extended version of the "summary slides" prepared for the meeting. Many of the findings summarized in this outline are illustrated by evidence and presented at the meeting (and included here); however, this evidence was presented to underscore the importance of epitope-specific regulation for studies of *in situ* and adoptive memory responses and consequently does not fully document the findings discussed. (For such documentation, we refer the reader to our published work.[6-12]

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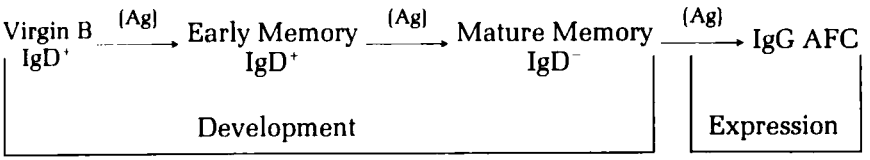
1. *B-cell events leading to IgG antibody production:*



- Mechanisms that regulate memory B-cell development control the potential for IgG antibody production.

- Mechanisms that regulate memory B-cell expression control which and how many of the memory B-cells present in a given animal actually give rise to AFC.

2. *Optimal development of memory B-cells requires:*

- Antigen reactive precursors (virgin B-cells)

- Carrier-primed T-cells or presentation of antigens in a form that stimulates the development of carrier-primed T-cells, e.g., on alum or in complete Freunds adjuvant (CFA)

- Support for the IgD^+ to IgD^- memory shift

3. *Optimal expression of memory B-cells (maximal IgG antibody production) for a given epitope requires:*

- Helper T-cells specific for the carrier on which the epitope is presented (or presentation of the antigen in a form that stimulates CTh development, e.g., on alum or in CFA)

- Active prevention of the induction of epitope-specific suppression for the response

4. *Epitope-specific suppression is induced by carrier-specific suppressor T-cells that mature shortly after priming and induce suppression for antibody production to individual epitopes on the carrier protein (unless antibody production to the epitopes is already in progress).*

5. *Rapid initiation of primary IgG responses (before CTs mature) tends to prevent the induction of suppression for antibody responses to individual epitopes. Thus:*

- Priming with a hapten-carrier conjugate enables primary and subsequent (anamnestic) IgG antibody responses to some but usually not all epitopes on the priming antigen.

- Suppression-induction protocols that induce strong suppression in animals that have not initiated an anti-epitope response are far less effective in animals already producing anti-epitope antibodies.

- Regulatory conditions that prevent initiation of selected IgG responses to epitopes on priming antigens (e.g., transient allotype suppression) result in the induction of a persistent suppression specific for those anti-epitope responses.

- The introduction of an epitope on a carrier to which the animal has previously been primed (carrier/hapten-carrier immunization) induces the epitope-specific system to selectively suppress IgG antibody responses to the "new" epitope (hapten).

6. *Induction of epitope-specific suppression by carrier/hapten-carrier immunization:*

- PROTOCOL. Immunize sequentially with a carrier protein and the "homologous" hapten-carrier conjugate, e.g., KLH/DNP-KLH; next, immunize with the hapten on an unrelated carrier molecule, e.g., DNP-CGG. (100 μg each antigen on alum).

- RESULT. Persistent and specific suppression of IgG anti-DNP antibody responses

7. *Response characteristics in KLH/DNP-KLH immunized animals:*

- Memory B-cells for all epitopes (including DNP) develop normally and are fully functional in adoptive assays.

- IgG antibody responses to the "new" epitope (DNP) on the priming carrier are specifically suppressed.

- Suppression persists after restimulation with DNP on an unrelated carrier (DNP-CGG) or on the priming carrier (DNP-KLH).

- Antibody responses to both carrier proteins proceed normally.

8. *Epitope-specific regulation is Igh-restricted. Thus selective suppression can be induced for:*

- The expression of memory B-cells committed to producing a given IgG isotype response to DNP, e.g., IgG2b or IgG3, (when carrier/hapten-carrier suppression-induction is suboptimal)

- The expression of memory B-cells committed to producing Igh-1b allotype responses to any of the epitopes on DNP-KLH (when young allotype heterozygotes are primed with DNP-KLH while allotype suppression is active)

9. *The epitope-specific system is a general regulatory mechanism (variable examined and result):*

• Epitope	
DNP, TNP	Suppression induced for both epitopes by carrier/hapten-carrier; suppression inducible for KLH epitopes by other protocols

• Carrier	
KLH, CGG, OVA, TGAL	All prime for suppression induction; some genetic restrictions (see below); 100 μg on alum sufficient
• Adjuvant	
KLH aqueous (2X)	+ + + + + *suppression induction*
KLH aqueous	+ + + + suppression induction
KLH on alum	+ + + suppression induction
KLH CFA	+ + + suppression induction
KLH on alum plus *B. pertussis*	No suppression induction
• Age	
KLH at 8 weeks to >6 months	Suppression equally strong at all ages
• Timing	
1 to 13 weeks between KLH and DNP-KLH	Suppression equally strong
• Persistance	
KLH/DNP-KLH then DNP-KLH or DNP-CGG up to 1 yr later	Suppression equally strong
• Carrier function genes (not in MHC)	
KLH with A/J or C57BL/10	Suppression induction by KLH/DNP-KLH impaired; (CGG/DNP-CGG OK)
• IR genes (MHC)	
TGAL/TNP-TGAL in C3H (H-2k) and C3H.SW (H-2b)	No interference with suppression induction; stronger suppression in "nonresponder" than in responder
• Mouse Strains	
BALB/c, BAB/14, SJL, SJA, C3H, C3H.SW, A/J, (SJL × BALB/c), C57BL/10, C57BL/6	Suppression inducible in all strains
• Chronic allotype suppression	
DNP-KLH prior to mid-life remission from Igh-1b allotype suppression	Igh-1b responses to DNP and KLH suppressed during remission; Igh-1b responses to new antigens OK; all other IgG responses OK
• Carrier-specific suppression	
KLH Ts or KLH-TsF plus DNP-KLH to nonirradiated recipients	Specific suppression induced for IgG anti-DNP

KLH-TsF (from thymus); DNP-KLH to irradiated recipients	Specific suppression induced for IgG anti-DNP
KLH-primed T-cells cells transferred; DNP-KLH at time of transfer	Suppression-induction favored for IgG anti-DNP in nonirradiated recipients; help for IgG anti-DNP favored in irradiated recipients

10. In situ antibody response failures can reflect interference either with the development or the expression of memory B-cells

Immunization(s)*		IgG2a anti-DNP Antibody in Serum	
KLH	DNP-KLH	*In situ* primary	Adoptive secondary† (donor B-cells + CTh)
		μg/ml (affinity)	μg/ml (affinity)
—	aqueous	3 (<1)	18 (<1)
alum	aqueous	5 (<1)	50 (8)
—	alum	35 (5)	73 (10)
alum	alum	<1 (<1)	75 (8)

* 100 μg indicated antigen at 9 and 3 (or 3) weeks prior to transfer.

†T-depleted spleen (B-cells), supplemented with KLH primed (nylon-passed) T-cell; 1 μg aqueous DNP-KLH to recipients; *in situ* anti-DNP measured 2 weeks after DNP-KLH immunization; adoptive anti-DNP 2 weeks after transfer; RIA assay (1); affinity = $Ka \times M^{-1} \times 10^6$ by RIA

11. T-cells in suppressed donors impair memory B-cell expression in adoptive assays

B-cell donor immunization(s)		Cells transferred to recipients	IgG2a anti-DNP μg/ml recipient serum
KLH	DNP-KLH	spleen (T + B)	32
KLH	DNP-KLH	T-depleted spleen + CTh*	104
—	DNP-KLH	spleen (T + B)	120
—	DNP-KLH	T-depleted spleen + CTh*	90

*KLH-primed T-cell supplement

100 μg each antigen i.p. on alum to donors; 1 μg aqueous DNP to (irradiated) recipients; IgG2a anti-DNP (μg/ml) in recipient serum 7 days after transfer (RIA).

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DISCUSSION OF THE PAPER

H. BAZIN: It's very amusing to see that your conclusion is very similar to the one people working with IgE in rodents have come to a long time ago. I think it was published about ten years ago that a very small dose of antigen can induce an IgE response in mice and I have published myself that the best immunization for IgE in rats or mice is to incorporate *B. pertussis* vaccine in the immunizing injection.

M. COOPER: It seems to me that it's pretty clear that if you stimulate an IgD bearing cell, whether with antigen or mitogens or anti-immunoglobulin, it ceases to express or it doesn't express as much IgD anymore. On that basis, since memory cells arise from stimulation of their immunoglobulin receptors, they wouldn't have IgD on them unless they have a capacity to stop making it and then expressing it later. Now that seems to me a fairly important question: Can a cell stop making IgD and then begin at another time later on?

F. R. BLATTNER: If you can sort of imagine the math that I put up on the first day where we have μ and δ close to one another and then γ way on down, and a transcript that begins at the D region and potentially can go all the way down to the most extremely distantly coded end of that transcript. There's a series of four or five transcription termination points, AATAAG sequences. The first level at which you can control the ratio of IgM to IgD is where the message is stopped. Thus, you could go from an IgM IgD positive cell to an IgM only cell just by elevating the number of enzyme molecules in the cell that clip near the AATAAG and cause a message to stop. The logical thing would be that the more you secrete of IgM, the more you express on the surface of the cell the IgM and the less IgD. I think that fits very beautifully with the type of data that we've got. But when a cell starts to secrete IgM, it stops secreting or expressing IgD on the surface. I'm not sure that has anything to do with memory. I think that has to do with just the virgin B-cell.

The next step is how you get into a γ on the surface. My prejudice is that the idea of making a messenger of 100 kilobases or more, which you would have to do to get down to γ 3, is unlikely. Honjo has published the only other way to go about it which is to delete the DNA between δ and γ. The rational way to express three classes at once, if that's really possible, would be by deleting from a point to the

right of δ to a point to the left of γ 3, or some other gamma. I think the thing you'd have to look at to be sure that was happening is to rule out the possibility of a transitory stage in which some messages hang around for an immunoglobulin that was expressed early. In such a transitory cell the DNA really isn't supporting all three of the Ig classes at once. That was the big problem we thought we had to answer in the case of the double-producing cells of μ and δ, that one needed a cell line that could be kept in culture long enough for any transitory message to be eliminated. I think what you are going to see happening is a deletion of the DNA from the switch side between μ and δ up to one of the γ. At that point, you really have a memory cell and it's going to be IgD negative.

LEE HERZENBERG: When we transfer δ-negative cells and then reseparate them after they had spent some time in a host, the δ-negative cells did not generate δ-positive memory. It doesn't totally answer the question because, of course, it could have come and gone. In any event, it seems to us as though once the cell is δ-negative, it remains δ-negative. When it's δ-positive, it's very likely to go to δ-negative on the next antigenic stimulation, but it can hang up in the δ-positive state under a suboptimal condition.

G. J. THORBECKE: We should distinguish between not being able to make something and preferring not to put it on the surface, or not to make it at all. It is very peculiar that germinal center cells, where, after all, some of this is going on, don't express anything. Not only no δ, but as Dr. Bazin pointed out, in germinal centers all surface immunoglobulin is extremely low, so it might not just be a matter of δ. It might be a matter of just not expressing very much immunoglobulin at all, temporarily, and perhaps going back to whatever they were doing before. Or maybe in the process they have switched and now they produce something else. Anyway, we should perhaps not say going to a δ^- stage and coming back to δ^+ is so impossible from that standpoint.

LEE HERZENBERG: I agree with your first statement absolutely. However, Eugene Butcher (and we) find that the germinal center cells early on have μ. They're bright, μ-bearing peanut agglutinin (PNA) positive cells.

THORBECKE: What I am referring to is described by Rose *et al.* and others. In sections, germinal centers aren't very strongly positive. But the problem is that there are some other PNA^+ cells also. So you can't just say that because you find some very strongly μ-positive PNA^+ cells that they were germinal center cells.

BLATTNER: The most likely sequence of events is that the first step deletes from the site between μ and δ down to γ. That makes the memory cell. And then the next step deletes from the classical Honjo-switch site, which is between J and μ, down to some point again in front of γ and that goes into an antibody secreting cell. I don't know whether you want this, but it's the simplest model at the molecular level. Now we have to see whether it fits the biological data.